



# Detection of cytogenetics abnormalities in chronic lymphocytic leukemia using FISH technique and their prognostic impact

O.M. Eid<sup>1</sup>, M.M. Eid<sup>1</sup>, H.F. Kayed<sup>1</sup>, W.M. Mahmoud<sup>1</sup>,  
S.S. Mousafa<sup>2</sup>, M.M. Ismail<sup>2</sup> and D.M. Abdeen<sup>1</sup>

<sup>1</sup>Human Cytogenetics Department, National Research Center, Cairo, Egypt, <sup>2</sup>Clinical and Chemical Pathology Department, Faculty of Medicine, Ain Shams University, Egypt

## Abstract

## Introduction

Chronic lymphocytic leukemia (CLL) is a clonal lymphoproliferative disorder characterized by progressive accumulation of morphologically and immunophenotypically mature lymphocytes. Characterization of genomic aberrations may help to understand the pathogenesis of CLL and may give prognostic information independent from conventional clinical markers for a risk-adapted management of CLL patients.

## Aim

The aim of the present study is to determine the most common cytogenetics abnormalities between patients with CLL and its prognostic impact.

## Patients and Methods

The present study was carried out on 20 adult patients presented with chronic lymphocytic leukemia. The patients were diagnosed on the basis of standard clinical (lymph node involvement and/or hepatosplenomegaly), hematological and immunophenotypic criteria for diagnosis of B-CLL. All cases were studied at the time of their diagnosis. FISH technique was successfully performed on PB samples

using CLL LSI probes for ATM (11q22) / GLI (12q13) and 13q14/ p53 (17p13).

## Results

For comparative statistical studies, the patients were divided into group I (patients with favorable outcome) and group II (patients with unfavorable outcome). All patients showed one or more cytogenetic abnormality with the prevalence of p53 in 16 patients out of 20 that perfectly correlated with the poor outcome of the patients. This is followed by deletion in the 13q14 and to a lesser extent deletion in ATM gene, but no one has exhibited amplification in the 12q13 locus.

## Conclusion

p53 deletion as a sole abnormality has a higher prognostic power than other cytogenetics abnormalities. The cytogenetics study using FISH panel for CLL patients in a complementary fashion to the other clinical and laboratory findings may overcome the pitfalls in the diagnosis and may also assess the assignment of therapeutic protocols for CLL patients according to the results of their cytogenetic analysis at the time of diagnosis.

## Keywords

*FISH, chronic lymphocytic leukemia, CLL, p53, cytogenetics, Egypt*

## Introduction

B-cell chronic lymphocytic leukemia (B-CLL) is the most common adult leukemia. It

is a haematopoietic neoplasm of B-lymphocytes found in the peripheral blood, bone marrow, and/or lymph nodes which is a clonal lymphoproliferative disorder characterized by progressive accumulation of morphologically and immunophenotypically mature lymphocytes. This disease is related to the accumulation of

Corresponding Author: Dr. Ola M. Eid, Human Cytogenetics Department, National Research Center, Cairo, Egypt. Tel No. 00201001775606. Email: olameid@hotmail.com

monoclonal B cells with the morphology of small mature lymphocytes <sup>(1)</sup>. CLL is one of the most common lymphoid malignancies that accounts for 30% of all leukemia <sup>(2)</sup>.

The presence of multiple chromosomal abnormalities in CLL has been implicated as an indicator of poor prognosis <sup>(3)</sup>. The most common cytogenetic anomalies in B-CLL involve chromosomes 6, 11, 12, 13, 14 and 17 <sup>(4)</sup>.

Deletion 13q is the most frequent genetic change in CLL, as it was reported in 40-65% of the disease <sup>(5)</sup>. The 13q14 deletions represent early clonal aberrations and suggest the presence of a tumor suppressor gene of which the loss or inactivation may be crucial to development of CLL <sup>(3)</sup>. Patients with deletion 13q14 as single aberration (no additional aberration detectable with a comprehensive FISH probe set) have the longest estimated median treatment-free interval and survival time (133 months) <sup>(6)</sup>. As a sole abnormality, deletion 13q14 is associated with good prognosis but additional abnormalities negate this favorable effect <sup>(7)</sup>.

Trisomy 12 is the second most common genetic abnormality identified in CLL when assessed by conventional cytogenetic analysis (CCA) <sup>(8)</sup>. The reported incidence in various studies is between 10% and 20%, either as an isolated finding or in combination with other genetic aberrations such as 6q del, 13q14 del and others. When assessed by interphase FISH, its incidence seems to be slightly higher in the range of 20% to 40% <sup>(3)</sup>.

Recurrent duplication of chromosome band 12q13-q21.2 was found in all cases, indicating that this region may contain the candidate oncogene(s) playing a pathogenic role in CLL <sup>(6)</sup>. Numerous studies have evaluated the prognostic effect of trisomy 12 in CLL and found that acquisition of this abnormality is associated with advanced-stage disease, shorter time to progression and significantly poor median survival rates <sup>(3)</sup>.

Structural aberrations of chromosome 11 are, by far, the most common recurring genetic event in a variety of lymphoproliferative disorders. Its incidence in CLL reported in various studies ranges from 12% to 25%. The region most

frequently deleted in CLL involves the long arm of chromosome 11 between bands q22 and q23. This region is rich in multiple genes that are involved in various hematologic malignancies, some of which are tumor suppressor genes such as ataxia telangiectasia mutated (ATM) <sup>(3)</sup>. The ATM gene which is located on 11q22, codes for a protein that acts upstream of p53 in the DNA damage response pathway <sup>(9)</sup>. Acquisition of del 11q22-23 is associated with disease progression and poor prognosis <sup>(10)</sup>.

The frequency of 17p13 deletion reported ranges between 9 and 15%. 17p13 locus host several genes including the p53 tumor suppressor gene. So, deletion of chromosome 17p might represent the most aggressive CLL subset displaying treatment failure <sup>(11)</sup>. Good prognosis CLL patients with deletions of the long arm of chromosome 13[del(13q)] as the sole aberration are opposed by patients with deletions of the short arm of chromosome 17 [del(17p)], who show poor prognosis <sup>(12)</sup>. Tumor protein 53 abnormalities consistently emerge as the most significant adverse prognostic factor in multivariate analyses of both prospective and retrospective studies in early and advanced CLL <sup>(13)</sup>.

Risk parameters were defined as prognostic markers that are more clearly related to the biology of B-CLL such as fluorescence *in situ* hybridization (FISH) <sup>(14)</sup>. So, the aim of this work was to detect cases of chronic lymphocytic leukemia with deletions of chromosome 13q14, 11q22 and/or p53 loci or amplification of chromosome 12p13 using FISH technique and to evaluate the prognosis values of these markers.

## Patients and Methods

This study was conducted on 20 adult patients who presented with B-CLL. All patients were subjected to the following: 1) Full history taking and thorough clinical examination with emphasis on lymphadenopathy, splenomegaly and hepatomegaly 2) Complete blood picture (CBC) 3) CT Imaging to detect internal lymphadenopathy and organomegaly 4) Bone marrow aspiration 5) Immunophenotyping of bone marrow or whole peripheral blood

6) FISH technique using fluorophore labeled locus specific identifier (LSI) dual color probes applied on peripheral blood or bone marrow samples for detection of 13q14 and/or p53 (17p13) deletions and of ATM (11q22) deletion and/or 12q13 amplifications 7) Patients were followed up after three cycle of chemotherapy by clinical evaluation of lymphadenopathy and organomegaly, CBC, CT imaging to assess the course of the disease and the outcome.

**Results and Discussion**

The results of this study are summarized in the Table 1.

Cytogenetic aberrations are considered to be among the major prognostic indicators for predicting survival of CLL patients. Conventional cytogenetic analysis (CCA) has been hampered by a low mitotic index of CLL cells in vitro, even if B-cell mitogens are used. Analysis of aberrant chromosomal regions with specific DNA probes, using FISH applied to interphase cells, has resulted in detection of clonal aberrations in 80% of CLL patients studied (15).

Specific genomic abnormalities, however such as 11q22 (ATM) and 17p13 (TP53) deletions,

trisomy chromosome 12 and loss of the 13q14 region, provide clinically meaningful prognostic information (16). They are known to be associated with prognostic impact in CLL patients, which is important for assignment of therapeutic protocol(17).

This study revealed cytogenetic abnormalities in all 20 cases (100%). Deletions involving only the 13q14 region were in 2/20 cases (10%). Deletions involving only the p53 region (17p13) were in 4/20 cases (20 %). Also, it revealed that del(13q14) was combined with 17p13 deletion in 7/20 cases (35%) and was combined with 11q22 in 2 cases (10%). While deletions involving these three regions together were found in 5 cases (25%), not one case showed 12q13 amplification.

In acceptance, Doneda et al.(18) found genomic aberrations in 100% of cases. Stilgenbauer et al. (19) found that, at initial analysis, 92% out of the cases had genomic aberrations. Haferlach et al.(20) found that cytogenetic aberrations were detected in 85.2% cases either by metaphase cytogenetics and/or interphase FISH. Quijano et al.(21) found that of overall B-CLL cases studied, 62% displayed one or more cytogenetic abnormalities. Also, Lu et al.(22) reported that

Case No.	p53 del	13q14 del	ATM del	12 amplification	Rai Staging	prognosis	Fate
1	30%	80%	0	0	I	Favorable	Alive
2	0	60%	0	0	II	Favorable	Alive
3	35%	55%	0	0	II	Favorable	Alive
4	13%	63%	0	0	II	Favorable	Alive
5	0	65%	0	0	III	Favorable	Alive
6	28%	30%	20%	0	III	Favorable	Alive
7	10%	90%	10%	0	IV	Favorable	Alive
8	100%	100%	0	0	I	Unfavorable	Alive
9	70%	0	0	0	II	Unfavorable	Alive
10	0	85%	35%	0	II	Unfavorable	Alive
11	50%	15%	0	0	II	Unfavorable	Alive
12	25%	33%	0	0	III	Unfavorable	Alive
13	100%	0	0	0	III	Unfavorable	Alive
14	35%	0	0	0	IV	Unfavorable	Alive
15	40%	35%	0	0	IV	Unfavorable	Alive
16	50%	26%	50%	0	IV	Unfavorable	Alive
17	50%	0	0	0	III	Unfavorable	Died
18	32%	48%	15%	0	III	Unfavorable	Died
19	0	80%	30%	0	IV	Unfavorable	Died
20	30%	70%	40%	0	IV	Unfavorable	Died
average	0-100	0-100	0-50	0			
mean	34.9	46.75	10.25	0			

**Table 1. Summary of results of the study**

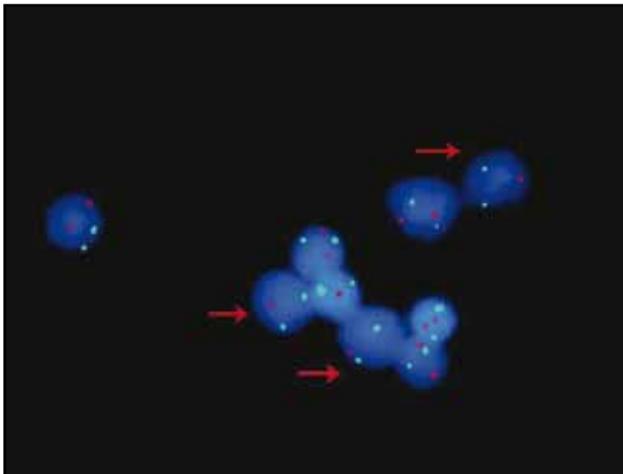
FISH analysis revealed that 55% of cases were positive for the probes used. This observed discrepancy could be attributed to the variation of the number of studied patients.

Trisomy 12 was assessed by interphase FISH by numerous groups determining the frequency of this aberration between 10% and 20%<sup>(23)</sup>. In 2000, Döhner et al<sup>(24)</sup> stated that FISH analysis trisomy 12 was the third most common chromosome aberration seen in 16% of 325 CLL cases. In our study, not one case showed 12q13 amplification. This observed discrepancy could be attributed to low number of presently studied patients.

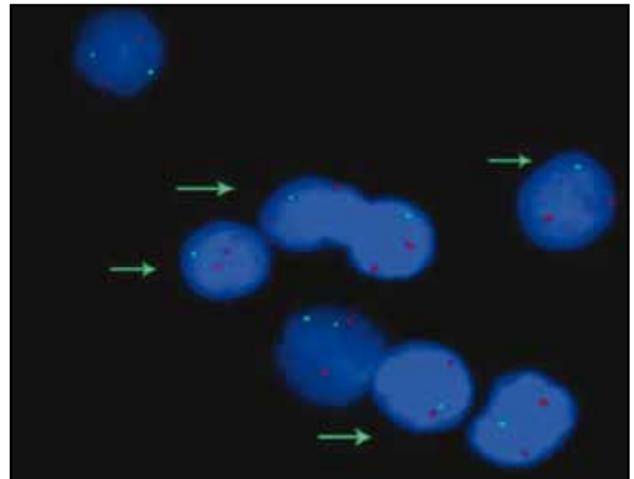
Del(13)(q14.3) is the most common isolated chromosome abnormality in CLL. It has good prognosis because of slow disease progression;

the median survival for a deletion 13q is 133 months<sup>(25)</sup>. Deletion of 13q14 region as a sole abnormality is associated with a good prognosis but additional abnormalities negate this favorable effect<sup>(7)</sup>.

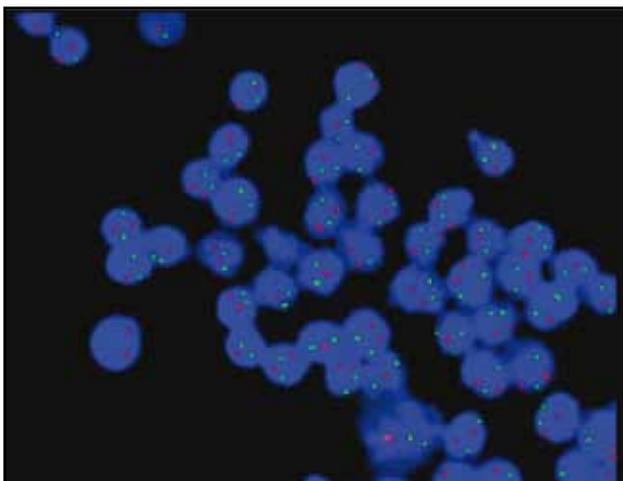
In the current study, positive B-CLL patients for only 13q14 deletion were 2/20 (10%) of cases (Figure 1). In the literature, studies showed a wide variability of results. Using the FISH technique, del 13q14 was detected in 18% of CLL patients by Jarosova et al.<sup>(26)</sup>, 25.3% by Durak et al.<sup>(27)</sup> 46% by Aoun et al.<sup>(28)</sup>, 66% by Bacher et al.<sup>(29)</sup>, 42.1% by Ripolle's et al.<sup>(30)</sup>, 45.8% by Reddy<sup>(31)</sup>, 31% by Mittal et al.<sup>(32)</sup>, 35% by Quijano et al.<sup>(21)</sup> and 42.2% by Xu et al.<sup>(33)</sup>. This observed discrepancy could be attributed to low number of presently studied patients.



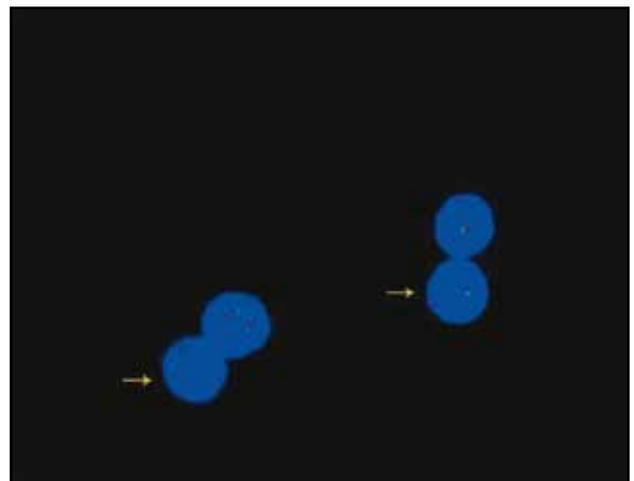
**Fig. 1:** Interphase FISH analysis showing positive 13q14 del denoted by the presence of one red signal and two green signals for the p53 region.



**Fig. 2:** Interphase FISH analysis showing positive p53 del denoted by the presence of one green signal and two red signals for the 13q14 region.



**Fig. 3:** Interphase FISH analysis showing positive 11q22 del denoted by one red signal and negative 12q13 amplification denoted by the two green signals.



**Fig. 4:** Interphase FISH analysis showing positive both del 13q14 and p53 denoted by one red signal and one green signal respectively.

In our study, no significant association between staging and 13q14 deletion was detected. Similarly, Starostik et al.<sup>(34)</sup>; Aoun et al.<sup>(28)</sup> found no significant differences in Rai stages at diagnosis were identified among the 13q14del subgroup. On the contrary, Karakosta et al.<sup>(35)</sup>; Döhner et al.<sup>(24)</sup> stated that it is usually found 13q14 del in patients with stage A disease according to the Binet system, suggesting an early clonal aberration in which the loss or inactivation of a tumor suppressor gene may be crucial for development of CLL. In acceptance, Ripolle's et al.<sup>(30)</sup> found that the majority of patients in initial stages (Rai stages 0-I) showed 13qdel.

Also, we found a high incidence of 13q14 deletion in cases with favorable outcome. In acceptance to this finding, Ripolle's et al.<sup>(30)</sup> found that deletion of 13q was strongly associated with favorable disease outcome showing better prognosis. Also, other reports stated that the patients with a deletion of 13q14.3 as single chromosome aberration had the longest estimated median treatment-free interval and survival times<sup>(17; 24; 30; 31; 36; 37)</sup>. On the contrary, Dewald et al.<sup>(4)</sup> and Sindelarova et al.<sup>(11)</sup> found no significant differences in distributions of FISH-identified chromosomal anomalies among patients with stable and progressive disease.

The strongest single predictor of bad prognosis is a deletion of chromosome 17p13, involving TP53, a tumor suppressor gene that is involved in cell cycling and cell death<sup>(38)</sup>. Inactivation of p53 by mutation or deletion occurs in approximately 50% of human cancers and is associated with genomic instability and resistance to chemotherapy<sup>(39)</sup>.

In the current study, positive B-CLL patients with only 17p13 deletion were 4/20 (20%) of cases (Figure 2). In the literature, studies using FISH technique, showed a wide variability of results. Sindelarova et al.<sup>(11)</sup> and Xu et al.<sup>(40)</sup> documented p53 gene deletion in 16% and 16.8% respectively of CLL patients. Abdel Salam et al.<sup>(41)</sup>; Dickinson et al.<sup>(42)</sup> and Gozzetti et al.<sup>(43)</sup> detected p53 deletion in 10% of CLL patients. Meanwhile, lower levels of detection were reported by other investigators: 8.7%<sup>(30)</sup>,

8%<sup>(21)</sup> and 3.4%<sup>(31)</sup>. This observed discrepancy between the present results and other studies could be attributed to different sample size and/or various studied ethnic groups.

In our study, there was no significant association detected between p53 and staging. On the contrary, Xu et al.<sup>(33)</sup> had shown that patients with advanced-stage disease had del (17p13) more frequently than patients at earlier stages. Also, Nelson et al.<sup>(44)</sup> found that the majority of cases in the poor prognostic FISH groups of isolated 17p del and isolated 11q del had advanced disease with Rai stage III or IV.

In the present study, all cases with 17p13 deletion only showed unfavorable outcome with one case of them died. Similarly, Xu et al.<sup>(33)</sup>, Durak et al.<sup>(27)</sup> and others<sup>(12, 24, 28, 30)</sup> documented that abnormalities of 17p are associated with a more rapid disease progression, poor outcome, drug resistance and a shorter survival time. On the contrary, Doneda et al.<sup>(18)</sup> found no correlation between the presence of TP53 deletions and clinical outcome or resistance to treatment.

ATM gene mutations - both point mutations and deletions - occur in a high proportion of cases of newly-diagnosed untreated CLL (24.6%), thus representing the most frequent unfavorable genetic anomaly in CLL. In view of the role played by ATM mutations on the behavior of CLL cells and progression of the disease, both deletions and point mutations should be considered in an optimal prognostic stratification of CLL patients and when deciding the management<sup>(45)</sup>. In our study, 7 cases (35%) showed 11q22 (ATM) deletion (Figure 3), 2 cases in combination with 13q14 deletion and 5 cases in combination with 13q14 and 17p13 deletions. The present study showed 5 cases out of the 7 cases (70%) have unfavorable outcome, in acceptance to Xu et al.<sup>(40)</sup> who stated that CLL patients with p53 and/or ATM gene deletion had poor therapeutic effect, and hence poor prognosis.

In the current study, deletions involving regions at 13q14 and 17p13 were found in 7 cases (35%) (Figure 4), deletions involving regions at 13q14 and 11q22 were found in 2 cases (10%) and deletions involving the three regions

together were found in 5 cases (25%). Shanafelt et al.(46) documented that 30% of cases had 2 aberrations and 6% of cases had 3 aberrations. Quijano et al.(21) found that around 12% of all B-CLL studied showed coexistence of two or more abnormalities in the same clone; these consisted of trisomy 12 associated with 13q-, 17p-, or 11q-, coexistence of 13q- with 17p- or 11q- and simultaneous presence of trisomy 12, 11q- and 13q- in the neoplastic B-cells.

Similarly, Sindelarova et al. (11) found, in accordance with other studies, combinations of different chromosomal changes as: trisomy12 with del13q14 and del13q14 with del17p13. They suggested that B-CLL is not genetically stable disease and clonal evolution is not uncommon in B-CLL patients. The prognostic importance of these combined changes remains to be clarified; however, it has been repeatedly stated that combination of chromosomal changes has an adverse effect on prognosis for survival.

In the present study, the percentages of patients with both deletions were 10%, 15%, 15% and 20% for Rai stages I, II, III and IV

respectively. While Dewald et al.(4) found that percentages of abnormal patients with 2 cytogenetic abnormalities or more were 32%, 45%, 38%, 57% and 55% for Rai stages 0, I, II, III and IV respectively. They stated that no consistent pattern in the distributions of either the FISH type of anomalies or the number of FISH anomalies among various Rai stages was apparent.

### Conclusion

Deletion of 13q14 region as a main abnormality is associated with a good prognosis but the presence of additional abnormalities such as p53 deletion or ATM deletion negate this favorable effect. While the strongest single predictor of bad prognosis is a deletion of chromosome 17p13, involving p53, a tumor suppressor gene that associated unfavorable outcome. As 13q deletion cases may transform because of accumulation of unfavorable secondary changes; follow up of del 13q cases using CLL FISH panel is suggested to detect secondary abnormalities with adverse prognosis.

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